Remarks

We respectfully request that the above-identified amendments entered into the file of the case. They are made to insert sequence ID numbers into the Specification and no new matter has been added.

An early action on the merits of the case is respectfully requested.

Respectfully submitted,

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In the Specification (Marked up version)

Please replace paragraph 008 (second paragraph on page 3) with the following:

[008] This invention relates to a mammalian secreted group XII $sPLA_2$ containing a potential Ca^{2+} binding segment (SEQ ID NO: 8) GCGSP.

Please replace paragraph 0010 (last paragraph bridging pages 3 and 4) with the following:

[0010] Fig. 1 represents the alignment of the amino acid sequences of $sPLA_2$s. In panel A, the full-length sequence of hGXII (SEQ ID NO: 2) is aligned with the amino acid sequences of mouse (SEQ ID NO: 10), rat (SEQ ID NO: 11), bovine (SEQ OD NO: 12) and *Xenopus* (SEQ ID NO: 13) GXII $sPLA_2$s (sequences were deduced from the alignment of different ESTs and from the BAC clone). For some $sPLA_2$s, the XX residues indicate that the sequence is partial. The *Arrowhead* indicates the predicted signal peptide cleavage site (32). The active site region containing catalytic site residues that are found in all $sPLA_2$s, and the putative Ca^{2+} binding segment (SEQ ID NO: 8) GCGSP are indicated. The level of identity between the mature protein sequence of hGXII and other GXII $sPLA_2$s is shown. Panel B shows alignment of the Ca^{2+}-binding and active site regions of hGXII (SEQ ID NO: 18) with a representative member of the four other structural classes of $sPLA_2$s (hGIB (SEQ ID NO: 14) for GI/II/V/X $sPLA_2$s, hGIII (SEQ ID NO: 15) for GIII $sPLA_2$s, Conodipine-M (SEQ ID NO: 16) for GIX $sPLA_2$, and Rice II (SEQ ID NO: 17) for GXI $sPLA_2$s).

Please replace the paragraph 0015 (last paragraph bridging pages 5 and 6) with the following:

[0015] Thus, the invention concerns a novel mammalian secreted group XII $sPLA_2$

wherein said enzyme contains a potential Ca^{2+} binding segment (SEQ ID NO: 8) GCGSP. The invention concerns more particularly a mammalian secreted group XII $sPLA_2$ comprising the sequence of amino acids under ~~SEQ ID N°2~~SEQ ID NO: 2. More particularly, the mammalian secreted group XII $sPLA_2$ is a human secreted group XII $sPLA_2$.

Please replace the 0034 (last paragraph bridging pages 14 and 15) with the following:

[0034] A blastp search with the amino acid sequence of hGXII $sPLA_2$ against the protein databases stored at the National Center for Biotechnology reveals matches to a variety of $sPLA_2$s from mammals, *C. elegans*, plants and animal venoms, suggesting that this protein belongs to the $sPLA_2$ family. The homology however appears to be weak (< 35% identity with blast scores lower than 35) and restricted to a short stretch of less than 60 amino acid residues containing the active site domain and the HD catalytic diad, indicating that the hGXII $sPLA_2$ is unique among all known $sPLA_2$s (Fig. 1B). The histidine of HD is thought to function as a general base to deprotonate a water molecule as it attacks the substrate ester carbonyl carbon, and the ß-carboxyl group of the adjacent aspartate coordinates directly to the catalytic Ca^{2+} cofactor (6,33). Except for 3 cysteines in the active site consensus sequence (SEQ ID NO: 9) CCXXHDXC which match those of other groups of $sPLA_2$s, the location of the other 11 cysteines residues in hGXII is distinct from that of other $sPLA_2$s (Fig. 1B). Since the structural arrangement of disulfides has been the main basis for designating the different $sPLA_2$ group numbers, the naming of the new $sPLA_2$ as hGXII seems appropriate.

Please replace paragraph 0035 (the paragraph bridging pages 15 and 16) with the following:

[0035] The homology between hGXII and all known $sPLA_2$s is so low that it is difficult to find the Ca^{2+} binding loop, which is usually highly conserved and provides 3 of the 4 amino acid ligands for the catalytic Ca^{2+} (34). All mammalian group I, II, V, and X $sPLA_2$s contain 19 amino acid residues between the most N-terminal residue that serves as a ligand to the active site Ca^{2+} (i.e. His-27 of hGIIA) and the catalytic histidine (i.e. His-47 of hGIIA). In contrast, the corresponding distances for hGIII and plant GXI $sPLA_2$s are 25 and 23 residues, respectively. hGXII contains a potential Ca^{2+} binding segment (SEQ ID NO: 8) GCGSP with 23 residues between the N-terminal glycine and the putative catalytic histidine as shown in Fig. 1. This segment is perfectly conserved among all of the GXII proteins found in gene databases. The x-ray structures of groups I, II, and III $sPLA_2$s reveal that the Ca^{2+} loop contains the consensus segment $X_1CG_1X_2G_2$. The backbone carbonyl oxygens of residues X_1, G_1, and G_2 coordinate to Ca^{2+}, and the backbone NH of G_1 is proposed to donate a hydrogen bond to the carbonyl oxygen of the enzyme-susceptible substrate ester (33,35). The fact that this residue is glycine in catalytically active $sPLA_2$s and that mutating this residue to serine lowers catalytic activity by about 10- to 20-fold (35) argues that steric bulk is poorly tolerated at this position. The putative Ca^{2+}-coordinating segment of hGXII shown in Fig. 1B fits the consensus sequence of other $sPLA_2$s with the exception that G_2 is a proline in hGXII. The prediction based on examination of the x-ray structures of $sPLA_2$s is that the hGXII Ca^{2+} binding segment should be functional. It contains G_1, and the backbone carbonyl of the C-terminal proline can coordinate to Ca^{2+} since its three extra methylenes, compared to glycine, are sterically allowed because of the location of this

residue on the enzyme's surface away from the substrate binding cavity. Interestingly, $sPLA_2$ isozymes with relatively low $sPLA_2$ activity from the venom of the banded krait also contain proline in place of G_2 (36).